REMARKS

Claims 6, 7 and 9-12 are pending in the instant application. Claims 6, 7 and 9-12 have been rejected. Claims 6 and 10 have been amended and claim 11 has been canceled. No new matter has been added by this amendment. Reconsideration is respectfully requested in light of the following remarks.

I. Objections

Withdrawal of the objection to the specification is acknowledged.

Claim 6 has been objected to for reciting the term "target cell". It is suggested that the use of the construct of claim 6 is in the method of claim 10 and therefore both claims should recite the term "cell". Applicant has amended the claims accordingly. Reconsideration and withdrawal of this objection is therefore respectfully requested.

II. Rejections Under 35 U.S.C. §112

Applicant acknowledges withdrawal of the rejections of base claim 10 and dependent claims for being unclear in reciting "the half-life of said fusion gene" and the relative terms "short", "rapid", and "longer".

Claims 6, 7 and 9-12 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to define the term "proliferation potential". To clarify, Applicant has amended claims 6 and 10, and claims dependent therefrom, to indicate that the rate of proliferation is being assessed using the claimed constructs and methods. Support for this amendment

can be found at page 16, lines 31-34, which states that the methods and constructs disclosed can be used to assess cell growth rates in evolutionarily divergent organisms from yeast to humans. In light of this amendment, withdrawal of this rejection is respectfully requested.

Applicant acknowledges withdrawal of the rejection of claims 10-12 for lack of written description based on the description of assessing the half-life of any fusion protein in a malignant cell.

Claims 10 and 12 remain rejected under 35 U.S.C. 112, first paragraph because the claims are directed to a large genus of methods using a large genus of DNA molecules encoding fusion proteins comprising any ubiquitin-like domain and any reporter protein. It is suggested that provision of nucleic acid molecules that encode Ubl of SEQ ID NO:2-11 is not sufficient for identifying all the species of the claimed DNA molecules encoding fusion proteins comprising any ubiquitin-like domain.

Applicant respectfully disagrees. However, in an earnest effort to facilitate the prosecution of the instant application, Applicant has amended claim 10 to clarify suitable UbL domains, namely amino acid sequences represented by SEQ ID NO:2-12, for use in the DNA construct of the claimed method. In light of this amendment, claim 11 has been canceled. Reconsideration and withdrawal of this rejection is therefore respectfully requested.

Claims 6, 7, and 9-12 have been rejected for lacking a sufficient description of assessing the proliferation potential of a cell. In particular, the Examiner suggests that the data presented in the instant disclosure is not a sufficient base for any routine determination as necessary in a claimed method. It is

suggested that degradation is of the fusion protein containing Ubl domain and a reporter proteins seems dependent upon the cell used, the details of the measurement and the fusion protein itself as evidenced by the data presented at page 32 (line 26), page 33 (line 34), Figure 10E (page 36, line 13), Figure 11B (page 37, line 6) and Figure 11A (page 17, line 13). In view of these data, it is suggested that one of ordinary skill in the art could not recognize that Applicant invented what is claimed. Applicant respectfully traverses this rejection.

Applicant respectfully disagrees with the Examiner's interpretation of the data presented at page 32, line 26 of the specification. This data indicates that unlike Rad23-HA (a protein having a UbL domain; *i.e.*, SEQ ID NO:3), R-βgal and Ub-P-βgal are efficiently degraded in both exponential and stationary phases of growth. R-βgal and Ub-P-βgal are fusion proteins of *ubiquitin itself* and βgal, not proteins having a UbL domain as set forth in the instant claims. See, for example, page 39, lines 15-18. Therefore, the stability of R-βgal and Ub-P-βgal proteins in exponential and stationary phases of growth is not indicative of the stability of claimed UbL domain-containing proteins under the same growth conditions.

Further, upon reading the Disclosure as a whole, one of skill in the art would appreciate that the utility of the claimed constructs and methods of the invention is in the assessment of the *general* rate of proliferation of cells. See page 16, lines 20-34. The data presented at page 33, line 34, indicates that *transient* growth arrest of exponentially growing cells does not effect the stability of a UbL domain-containing protein in that said protein is not detected for up to one hour after the cell

has been arrested. Thus, it would be clear to the skilled artisan that this data was the result of the analysis of an artificial growth state and not representative of the intended utility of the inventive construct and methods for assessing the general rate of proliferation of cells.

Likewise, the skilled artisan would appreciate that the analysis of the stability of a UbL domain-containing protein in ufd5Δ, cim5-1 and pre1-1 pre2-2 mutant cells was to demonstrate that UbL degradation required the ATP-dependent proteasome pathway. These mutants are conditional mutants, which under standard conditions would not be viable; cim5-1 is temperature sensitive mutant, whereas ufd5Δ and pre1-1 pre2-2 are extremely sick and inviable following stress. In general, it is difficult to assess the rate of proliferation of a cell with defects in this pathway, because such mutations generally result in lethality. Most mutations in this pathway are gain-of-function, or altered function and, in fact, proteosomic proteins have been found to be upregulated in hyperproliferative cells. See, *e.g.*, Kanayama et al. (1991) *Cancer Res.* 51:6677-85 and Baracos, et al. (1995) *Am. J. Physiol.* 268(5 Pt 1):E996-1006; abstracts enclosed herewith. Thus, one of ordinary skill in the art would recognize that the ufd5Δ, cim5-1 and pre1-1 pre2-2 mutant cells are not representative of the types of cells whose rate of proliferation would be assessed using the DNA constructs of the instant invention. Accordingly, Applicant has provided a sufficient description which would clearly allow persons of ordinary skill in the art to recognize that Applicant invented what is claimed. It is therefore respectfully requested that this rejection be withdrawn.

Claims 6-7 and 9-12 have been rejected under 35 U.S.C. §112, first paragraph, because the specification, while being enabling for degradation of $Rad23^{1-369}$, Rad23-HA and Ubl^{R23}-lacZ with 0-30 min. after labeling when the labeling is performed in some exponentially growth yeast transformants (Figures 7 and 9), does not reasonably provide enablement for assessment of a proliferative potential of any cell using any fused DNA encoding a protein consisting of any Ubl domain and a reporter protein. It is acknowledged that the art of construction of DNA molecules encoding fusion proteins is highly developed and skill of the artisan is high; however, it is suggested that because the structure of the claimed fusion protein to be used, and the method of use itself, are lacking enabling description based upon the same arguments set forth in the rejection for lack of written description, one skilled in the art is forced to perform undue experimentation with low probability of success. It is further suggested that the degradation of any fusion protein in any multiplying cell was not disclosed and the half-life of any particular construct is dependent upon its structure as evidenced by the teachings of U.S. Patent No. 5,132,213. Applicant respectfully disagrees.

Applicant has established that a correlation exists between cell proliferation and stability of Ubl domain-containing proteins. Applicants have provided *multiple* examples of Ubl domain-containing proteins (*i.e.*, UbL^{DSK}, UbL^{R23}, UbL^{HRB} fused to reporters such as GST and β-gal) and demonstrate that these fusion proteins bind to the proteasome (see pages 41 and 42), wherein the binding is more favorable in actively growing cells (*e.g.*, GST-UbL^{DSK}; see page 44, lines 20-22), leading to an

decrease in the stability of the fusion protein in actively growing cells (*e.g.*, UbL^{R23}-βgal; see pages 32-33). Further, as Applicant has indicated *supra,* the data presented at pages 32-33, Figure 10E, Figure 11B and Figure 11A represents the analysis of the pathway involved in degradation of a Ubl domain-containing protein and would be interpreted by one of skill in the art as falling outside of the scope of instant claims as R-βgal and Ub-P-βgal proteins are fusion proteins of ubiquitin itself; defects in ufd5, cim5-1 and pre1-1 pre2-2 generally result in cell lethality; and transient growth arrest of exponentially growing cells is not indicative of the general rate of proliferation of a cell. Further, while the teachings of U.S. Patent No. 5,132,213 indicate that fusion protein degradation may be dependent upon the link between the a reporter and a ubiquitin, the teachings of this reference focus on ubiquitin itself and not Ubl domains. Therefore, these teachings may not be reliably used to establish a correlation between structure and stability of a Ubl domain-containing protein. Accordingly, because Applicant has provided multiple examples of DNA constructs encoding fusions between a UbL domain and a reporter molecule and disclosed the utility of these constructs for assessing the rate of proliferation of a cell, Applicant has satisfied the enablement requirement set forth under 35 U.S.C. §112, first paragraph. Therefore, it is respectfully requested that this rejection be reconsidered and withdrawn.

III. Rejection Under 35 U.S.C. §102

Applicant acknowledges the withdrawal of this rejection of claims 6, 7 and 9.

IV. Conclusion

The Applicants believe that the foregoing comprises a full and complete response to the Office Action of record. Accordingly, favorable reconsideration and subsequent allowance of the pending claims is earnestly solicited.

Respectfully submitted,

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ARTICLES

Activation of the ATP-ubiquitin-proteasome pathway in skeletal muscle of cachectic rats bearing a hepatoma

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Rats implanted with Yoshida ascites hepatoma (YAH) show a rapid and selective loss of muscle protein due mainly to a marked increase (63-95%) in the rate of protein degradation (compared with rates in muscles of pair-fed controls). To define which proteolytic pathways contribute to this increase, epitrochlearis muscles from YAH-bearing and control rats were incubated under conditions that modify different proteolytic systems. Overall proteolysis in either group of rats was not affected by removal of Ca2+ or by blocking the Ca(2+)-dependent proteolytic system. Inhibition of lysosomal function with methylamine reduced proteolysis (-12%) in muscles from YAH-bearing rats, but not in muscles of pair-fed rats. When ATP production was also inhibited, the remaining accelerated proteolysis in muscles of tumor-bearing rats fell to control levels. Muscles of YAH-bearing rats showed increased levels of ubiquitin-conjugated proteins and a 27-kDa proteasome subunit in Western blot analysis. Levels of mRNA encoding components of proteolytic systems were quantitated using Northern hybridization analysis. Although their total RNA content decreased 20-38%, pale muscles of YAH-bearing rats showed increased levels of ubiquitin mRNA (590-880%) and mRNA for multiple subunits of the proteasome (100-215%). Liver, kidney, heart, and brain showed no weight loss and no change in these mRNA species. Muscles of YAH-bearing rats also showed small increases (30-40%) in mRNA for cathepsins B and D, but not for calpain I or heat shock protein 70. Our findings suggest that accelerated muscle proteolysis and muscle wasting in tumor-bearing rats result primarily from activation of the ATP-dependent pathway involving ubiquitin and the proteasome.

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1: Cancer Res. 1991 Dec 15;51(24):6677-85. Related Articles, Links

Changes in expressions of proteasome and ubiquitin genes in human renal cancer cells.

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Proteasomes and ubiquitin (Ub) are essential components of the energy-dependent, nonlysosomal proteolytic pathway. To clarify the physiological role of this proteasome/Ub-dependent pathway, we meaured the levels of expressions of proteasomes and Ub in human renal cancers by Northern blot and immunochemical analyses. The mRNAs for two of the multiple subunits of proteasomes, C2 and C9, were expressed at abnormally high levels in most neoplastic lesions of patients with various primary renal cell carcinomas and in all renal cancer cell lines examined. However, no significant difference was found by enzyme immunoassay in the proteasomal contents of cancerous and normal parts of the kidney. The levels of mRNAs for the subunits of proteasomes were high in rapidly proliferating renal cells and appeared to be correlated with the activities of these cells for proteasome synthesis, but the cellular contents of proteasomes in these cells were normal, suggesting rapid turnover of proteasomes in rapidly proliferating cancer cells. Consistent with the increased expressions of proteasomal mRNAs, the expressions of three Ub genes, mono-UbA80, mono-UbA52, and poly-UbC, were found to be greatly increased in these renal cancer cells. Immunohistochemical staining of normal kidney showed that the levels of both proteasomes and Ub were high in cells of renal tubules and collecting ducts, but low in the glomerulus. The levels of both proteins appeared to be considerably increased in the nuclei of granular and clear carcinoma cells of the kidney. Moreover, the profiles of cellular proteins conjugated with Ub in normal kidney tissues were different from those in cancerous parts of the kidney and in established renal cancer cells. These results suggest that the proteasome- and ubiquitin-mediated system is functionally involved in the cancerous state in human kidney.

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3) Abstract for Kanayama et al. (1991) *Cancer Res.* 51:6677-85;

4) Abstract for Baracos, et al. (1995) *Am. J. Physiol.* 268(5 Pt 1):E996-1006; and

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